REMARKS

Status of the Application:

This paper is filed in response to the Office Action mailed on March 13, 2007 (hereinafter, the "Office Action"). At the time the Office Action was mailed, claims 1-11 were pending in the application. Claims 12-14 have been conditionally withdrawn without prejudice, subject to the restriction requirement in the Office Action dated December 14, 2006. In the instant response, claims 1, 6, and 8 have been amended, claim 7 has been cancelled, and claims 15 and 16 have been added. Support for claim 15 may be found in the specification, e.g., on page 21, lines 26-32. Support for claim 16 may be found, e.g., on page 20, line 22 to page 21, line 25. Therefore, upon entry of the instant amendment, claims 1-6, 8-11, and 15-16 will be before the Examiner for consideration.

Drawings:

The Office Action at page 2 states that the drawings (FIGS. 2, 3, and 6) as filed do not comply with 37 C.F.R. § 1.84(U)(1) because partial views of a drawing which are intended to form one complete view must be identified by the same number followed by a capital letter.

In response, Applicants submit herewith amended FIGS. 2, 3, and 6 of the drawings. It is believed that the drawings as amended are in compliance with 37 C.F.R. § 1.84(U)(1).

Specification:

Consistent with the requested amendments to rename various views in FIGS. 2, 3, and 6, the appropriate paragraphs in the specification (Brief Description of Drawings and Examples) have been amended to reflect the changes (see above section entitled "In the Specification").

Rejections Under 35 U.S.C. § 112, second paragraph:

Claims 1-11 are rejected under 35 U.S.C. § 112, second paragraph as being indefinite for allegedly failing to point out and distinctly claim the subject matter which Applicant regards as the invention.

More particularly, claims 5 and 6 are rejected as being vague and indefinite because the meaning of the term "tissue-restoring" cannot be ascertained. According to the Office Action, it is not clear whether the term "restoring" denotes one of the following processes, viz. "maintaining, culturing, stabilizing, preserving...etc.," and hence the metes and bounds of the claim cannot be determined.

As a preliminary matter, Applicants note that claim 5 does not recite the term "tissue-restoring;" accordingly, it is respectfully submitted that the amendments and arguments should not pertain to claim 5, but only to claim 6.

In response, Applicants direct the Examiner's attention to the specification, at page 15, lines 11-19 (reproduced below), in which the term "tissue-restoring" is described:

> For example, at the iris-tissue-restoring stage P5, after the reaction of the enzyme treatment stage P4, the iris tissue is allowed to react for 30 to 60 minutes in a culture medium containing a commercially available fetal calf serum so as to restore the iris tissue. The serum-containing culture medium and reagent used at the iris-tissue-restoring-treatment stage P5 are not particularly limited. It is possible to use a publicly known conventional culture medium and reagent which make it possible to recover weakened iris tissue.
>
> (Emphasis added)

Consistent with the above-recited language used in the specification, claim 6 has been amended to recite the phrase "by using a culture medium containing serum, the iris tissue weakened by the enzyme treatment."

In view of the amendment, Applicants submit that the meaning of claim 6 is clear and definite, and accordingly respectfully request reconsideration and withdrawal of the rejection of claim 6 under 35 U.S.C. § 112, second paragraph.

Claim 1 is rejected under 35 U.S.C. § 112, second paragraph, as being incomplete for allegedly omitting essential steps, such omission amounting to a gap

between the steps. According to the Office Action, a step is omitted that indicates the culture conditions for obtaining tissue cells.

Claim 1 has been amended to recite the step of obtaining tissue cells from the pluripotent stem cells "by differentiating the pluripotent stem cells into one or more types of tissue cells by culturing the pluripotent stem cells under differentiation inducing condition." Support for the language in the recited phrase may be found in claim 7 as originally filed (and presently cancelled herein). By this amendment, Applicants submit that the alleged lacking step has been added to claim 1, and that claim 1 as presented herein is not indefinite.

Claims 2-4 and 7-11 are rejected as indefinite for being dependent from indefinite claims (1 and 6). In view of the amendments to claims 1 and 6 presented herein, it is respectfully submitted that claims 2-4 and 7-11 now depend from claims that do not suffer from indefiniteness. Accordingly, Applicants respectfully request that the rejection of claims 2-4 and 7-11 be reconsidered, and withdrawn.

Rejections Under 35 U.S.C. § 112, first paragraph:

1. Enablement.

Claims 1-11 are rejected under 35 U.S.C. § 112, first paragraph, for alleged lack of enablement because, according to the Office Action, the specification, while being enabling for a method of producing stem cells expressing Oct-3/4 and cardiac genes GATA4 and Nkx2.5, by selectively culturing iris pigment epithelial cells, does not reasonably provide enablement for a method for producing pluripotent cells that are tridermic differentiatable from the iris pigment epithelial (IPE) cells.

Applicants respectfully disagree, and direct the Examiner's attention to description in the specification that demonstrates Applicants' ability to detect markers of all three germ layers (endoderm, mesoderm and ectoderm) in IPE cells grown under the described culture conditions. The specification, starting on page 11, line 18 through to page 13, line 4 provides a description of a method of inducing differentiation of the stem cells into various types of tissue cells in an "embryoid body."

Further, Example 2 (starting on page 20, line 22) provides details of studies of IPE cells cultured for three days in serum-free medium as described, and thereafter for 1-2 months in floated coagulated mass culture, in one of three different culture media

(media described on page 21, lines 1-4). Following such culture, and RNA extraction from the resulting aggregates, RT-PCR was used to examine for the presence or absence of transcripts of markers of each of the three germ cell layers, (i.e., fetoprotein-α (an endodermal marker); myosin and MEF2 (mesodermal markers) and pax 6 and tubulin J (ectodermal markers)).

As described in the specification, e.g., on page 21, lines 5-13, the results showed that under any one of the three conditions, expression was observed of the marker genes representing each germ layer, demonstrating that the "obtained aggregates include cells differentiated into all three types of tridermic tissue." These results are further summarized on page 21, lines 14-25, which states:

> Although the iris pigmented epithelial cells are ectodermal cells, the present example showed that it is possible to allow stem cells derived from iris pigmented epithelial cells of an animal to be differentiated into mesodermal cells and endodermal cells as well as ectodermal cells. That is, the present embodiment showed that the cells obtained by the stem-cell-producing step have tridermic differentiation potency and can be differentiated into any one of a mesoderm, an endoderm, and an ectoderm.

In view of the above description, and the high level of skill in the field of stem cell culture, Applicants respectfully submit that one of skill in the art, reading the specification, would know how to make and use the invention as claimed, and accordingly request reconsideration, and withdrawal, of the rejection of claim 1, and claims dependent thereon, for lack of enablement.

2. Written Description.

Claims 1-11 are also rejected under 35 U.S.C. §112, first paragraph, for lack of written description. According to the Office Action, the description of mesodermal cells (cardiac myocytes) and ectodermal cells (iris) does not provide adequate written description of an entire genus of tissues and cells that are tridermic differentiable.

Applicants respectfully disagree, having demonstrated, as discussed above, that the embryoid bodies of the invention possess tridermic differentiation potential as evidenced by the expression of markers of each of the germ cell layers, and further

exemplified by the demonstration of formation of cardiac myocytes. To further claim the invention, claim 15 has been added, separately reciting myocardial cells.

In view of the above arguments, Applicants respectfully submit that one of skill in the art, reading the specification, would conclude that the Applicants were in possession of the invention, and accordingly request reconsideration, and withdrawal, of the rejection of claim 1, and claims dependent thereon, for lack of written description.

Rejections Under 35 U.S.C. § 103:

Claims 1-11 are rejected under 35 U.S.C. § 103 as being unpatentable over Kosaka et al. (Exp. Cell Res. 245:245-251, 1998; "Kosaka") and Haruta et al. (Nature Neurosci. 4:1163-1164, 2001; "Haruta") in view of Reynolds and Weiss (Science 255:1701-1710, 1992; "Reynolds").

Applicant respectfully disagree, and submit that Kosaka teaches that iris pigment epithelial cells of chicks can be successfully isolated and cultured. Haruta teaches that by adding a modification to the method of Kosaka, iris-derived cells of mammals can be isolated and cultured. However, neither the Kosaka or Haruta reference teaches or suggests a floated coagulated mass culturing technique.

The deficiencies of Kosaka and Haruta are not remedied by Reynolds. The latter reference teaches that neuroepithelial stem cells can be derived from cells isolated from the striatum of mouse brain and differentiated into neurons and astrocytes. Moreover, Reynolds teaches that neural stem cells can be proliferated as a spherical aggregate of cells (neurosphere)

The Office Action (page 13, paragraph 36) states that the person of ordinary skill in the art would have been motivated to use the Reynolds culture technique ("suggestive of the floated coagulated mass culturing technique using neurospheres") for cell culture of iris PE, because "this would facilitate the selection of a specific cell type aggregate by antibody immuno-staining." The Examiner further reasons that one of skill in the art would have expected success by making the hypothetical combination of the teachings of the references because the method of culturing neurospheres was well accepted in the art.

Applicants respectfully disagree for the following reasons. Reynolds fails to disclose that a floated coagulated mass culturing technique can be successfully used

for culturing any type of cells other than neural cells. Reynolds does not teach that any type of cell can be used to form a neurosphere, and certainly Reynolds does not teach or suggest that a floated coagulated mass culturing technique can be used to culture iris pigmented epithelial cells isolated from the eye. Reynolds does not even mention IPE cells, or in fact any cells of the eye at all. Given the teachings of Reynolds, one of skill in the art would have no reason to expect that any type of cell other than a neural cell would form a neurosphere under the Reynolds conditions, and particularly would have no reason to expect that an IPE cell (an ectodermal cell, not a neural cell) would behave like a neural stem cell *in vitro*, or would form a structure like a neurosphere when grown under conditions used by Reynolds

Therefore, Applicants respectfully submit that there exists no motivation or suggestion for one of skill in the art to combine the disclosure of Kosaka and Haruta (teaching IPE isolation) with Reynolds (teaching neurosphere culture method), to arrive at the invention as claimed. Accordingly, it is submitted that the Office Action fails to make out a *prima facie* case of obviousness

Furthermore, as described in the specification it is Applicants who have made the unexpected discovery that it is possible, upon culturing IPE cells as described under a floated coagulated mass culture technique, to selectively cultivate stem cells from IPE that have the potential to differentiate into various types of tissue cells representing each of the three germ cell layers.

In view of these arguments, it is submitted that the rejection of claims 1-11 as being obvious over the combination of the cited references has been overcome, and respectfully request reconsideration, and withdrawal of the rejections.

Conclusion:

In view of the amendments and arguments presented herein, Applicants submit that the claims are in condition for allowance Early and favorable action is requested.

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Respectfully submitted,

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FIG. 2

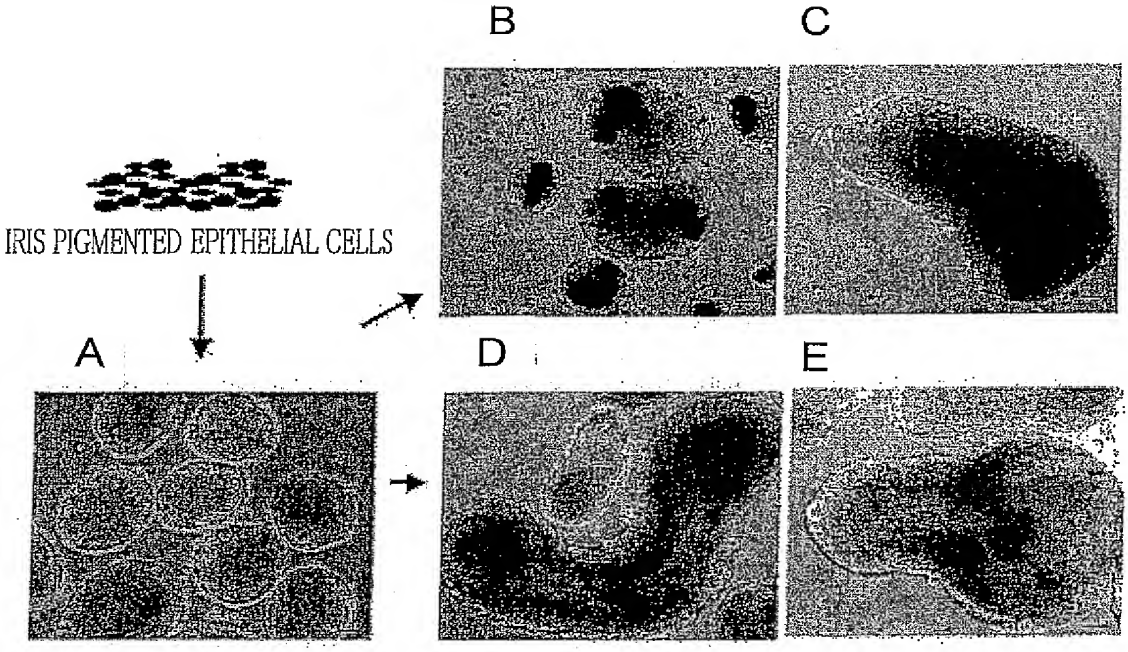


FIG. 3 A

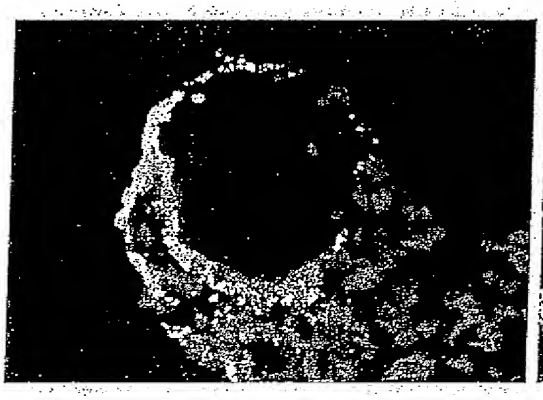


FIG. 3 B

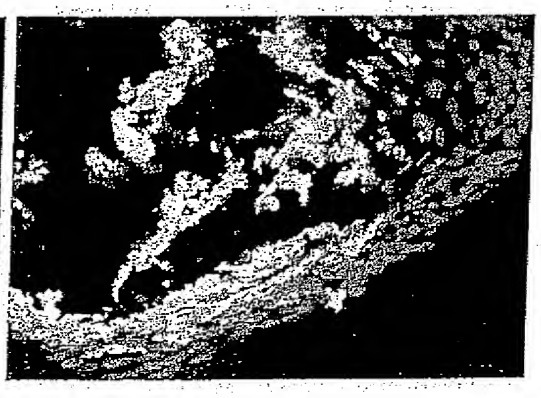
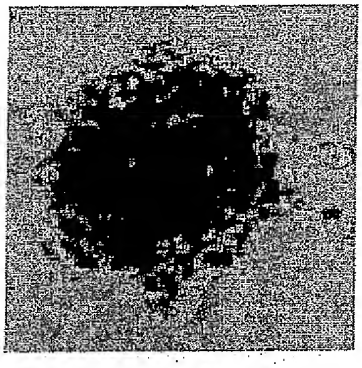
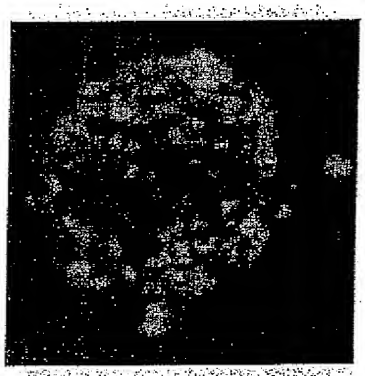


FIG. 6 A

11-DAY-OLD RAT





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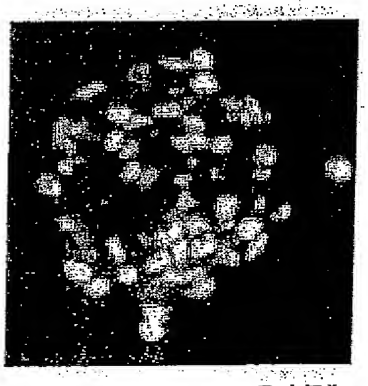

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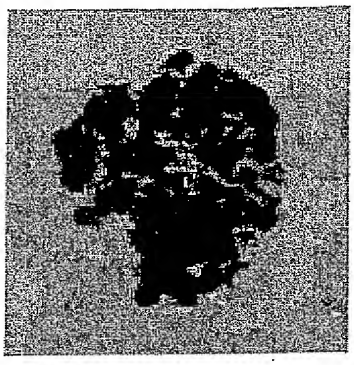
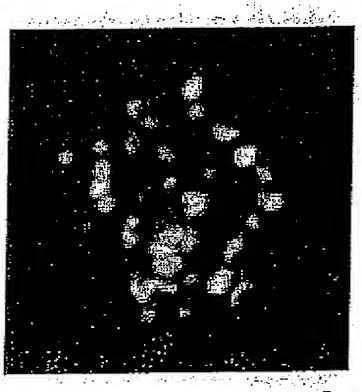
FIG. 6 B

3-WEEK-OLD RAT



Oct-3/4



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